

catch sizes for the different traps over time was significantly different ( $P < 0.05$ ).

The dry ice-baited silhouette trap sampled significantly greater numbers of *S. arcticum* and *S. verecundum* than any of the other traps examined (Table 2). The CO<sub>2</sub> generator trap sampled greater numbers of these two species than the unbaited silhouette trap. The trap type  $\times$  day interaction was significant for *S. arcticum* and *S. verecundum*. For *S. decorum*, the dry ice-baited silhouette trap sampled a significantly greater number of flies than the unbaited silhouette trap. There were no significant differences in the numbers of *S. vittatum* and *S. venustum* caught in any of the traps. Only 366 *S. vittatum* and 186 *S. venustum* were collected over 12 days.

*Simulium arcticum* is the major black fly pest species of cattle in Alberta (Fredeen 1969). Traditionally, control programs for black flies in Alberta have centered around monitoring population changes for *S. arcticum*. The CO<sub>2</sub> generator-baited silhouette trap collected fewer *S. arcticum* than the dry ice-baited trap, but more *S. arcticum* than the unbaited silhouette trap. However, the trends in the numbers of *S. arcticum* over time were significantly different between the dry ice- and CO<sub>2</sub> generator-baited silhouette traps. In this study, the CO<sub>2</sub> generator was not as effective as dry ice for monitoring black fly populations using the silhouette trap. However, the CO<sub>2</sub> generator could be useful in remote areas where dry ice is not available.

#### References Cited

- Fredeen, F. J. H. 1969. Outbreaks of the black fly *Simulium arcticum* Malloch in Alberta. *Quaest. Entomol.* 5:341-372.
- Fredeen, F. J. H. 1977. A review of the economic importance of black flies (Simuliidae) in Canada. *Quaest. Entomol.* 13:219-229.
- Fredeen, F. J. H. 1981. Keys to the black flies (Simuliidae) of the Saskatchewan River in Saskatchewan. *Quaest. Entomol.* 17:189-210.
- Haufe, W. O. and G. C. R. Croome. 1980. Control of black flies in the Athabasca River. *Techn. Rep., Pollution Control Div. Alberta Environment, Edmonton.* 241 pp.
- Peterson, B. V. 1960. The Simuliidae (Diptera) of Utah, Part I. Keys, original citations, types and distribution. *Great Basin Nat.* 20:81-104.
- Shemanchuk, J. A. 1981. Repellent action of permethrin, cypermethrin and resmethrin against black flies in the Athabasca River. *Tech. Rep., Sci.* 12:412-416.
- Shipp, J. L. 1985. Comparison of silhouette, sticky, and suction traps with and without dry-ice bait for sampling black flies (Diptera: Simuliidae) in central Alberta. *Can. Entomol.* 117:113-117.
- Steel, R. G. D. and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc. Toronto. 481 pp.
- Weast, R. C. 1972. Handbook of chemistry and physics. The Chemical Rubber Co., Ohio.

#### TECHNIQUE FOR VOLUMETRICALLY MEASURING EGGS OF *CULEX QUINQUEFASCIATUS*

JAMES A. THOMAS AND DONALD L. BAILEY

Insects Affecting Man and Animals Research Laboratory, USDA, Agricultural Research Service, Gainesville, FL 32604

It has been demonstrated that eggs of some *Anopheles* species can be dried and volumetrically measured (Dame et al. 1978, Bailey et al. 1979). This development greatly increased the efficiency of mass rearing *Anopheles albimanus* Weidemann in El Salvador (Bailey et al. 1980). In the past this method has not been used with *Culex* species because mosquitoes of this genus lay their eggs in rafts. This paper reports the development of a suitable technique for drying egg rafts of *Culex quinquefasciatus* Say, thus allowing volumetric egg measurements to be made for setting rearing trays.

Freshly collected egg rafts, not more than 24 hr old, were placed in a polyethylene cup 10 cm diam. with the bottom replaced with organdy cloth for drying in an apparatus previously described by Dame et al. (1978). Immediately after drying for ca. 20 min, the rafts were transferred to a 120-ml empty vial and individual eggs were separated by lightly pressing the rafts against the wall of the vial with an artist's brush (#4 Wilton Flat, Windsor, NJ). The eggs were then sifted through a screen (20 mesh/cm insert in a modified bottle cap) and volumetrically measured into graduated pipettes through a funnel. To determine if this handling technique caused any adverse effects on further development, the eggs were placed in rearing trays (56  $\times$  43  $\times$  7.5 cm) containing 3 liters of water and the larvae were reared to the pupal stage using procedures similar to those reported by Dame et al. (1978) in their paper on rearing *An. albimanus*. Pupal harvests were made on days 5 and 6 after trays were set using the ice water technique described by Hazard (1967). Reported here are the results of 3 replicates, each of which included 3 trays at 3 test densities of dried eggs (0.04, 0.05 or 0.06 ml per tray). Controls consisted of 10 nondried, intact egg

Table 1. Effects of drying and volumetrically measuring *Culex quinquefasciatus* eggs on their further development.

Parameter	Volume of dried eggs/tray (ml)			Control (10 rafts)
	0.04	0.05	0.06	
No. eggs/sample	1369 ± 86	1711 ± 107	2054 ± 129	1674 ± 22
Egg hatch (%)	92.9 ± 3.3	89.0 ± 6.7	87.7 ± 9.2	83.0 ± 15.1
No. pupae/tray (2 harvests)	1132 ± 439	1115 ± 862	980 ± 1073	1047 ± 752
No. of larvae failing to pupate after 2 harvests	208 ± 153	871 ± 882	1950 ± 1109	795 ± 327
Adult emergence (%)	98.01 ± 1.7	98.3 ± 1.2	96.1 ± 2.2	95.3 ± 4.4
Adult survival after 7 days (%)	97.0 ± 3.0	97.3 ± 1.2	98.3 ± 0.6	99.0 ± 1.0

rafts per tray. The parameters observed were the average number of eggs contained in the test samples, the percentage egg hatch, total number of pupae produced per rearing tray, number of larvae that failed to pupate (analyzed by chi-square), adult emergence and adult survival after 7 days (analyzed by student-*t* test).

Table 1 shows the results of these tests. The average numbers of eggs contained in the test samples were  $1,369 \pm 86$  for 0.04 ml,  $1,711 \pm 107$  for 0.05 ml,  $2,054 \pm 129$  for 0.06 ml and  $1,674 \pm 22$  for the controls. Of the 3 volumetric samples 0.05 ml more closely approximated the number of eggs in 10 rafts. There was no significant difference in the mean percentage hatch among the four treatments, although the sample variability was much less with 0.04 ml. Increase in variability was directly correlated to an increase in volume, but the controls were the most variable.

There was no statistically significant difference in pupal production between treatments, demonstrating that the lower volume of eggs was more economical. If equivalent numbers of pupae can be produced by using a lower volume of eggs, then the cost of rearing would be reduced by reducing the size of the adult colony required for egg production. Supporting evidence is shown by the data on unsuccessful pupation, which increased from 208 unharvested larvae at 0.04 ml to 1950 at 0.06 ml ( $9.4\times$  increase). The number of larvae remaining after the second harvest in sets made with 0.05 ml, 0.06 ml and the controls were all significantly higher than the sets made with 0.04 ml ( $P = 0.05$ ).

To assess the possible deleterious effects of egg drying on later developmental stages, tests were conducted to compare this treatment with past stock colony standards. The percentage adult emergence and adult survival after 7 days were monitored in a holding cage ( $12.5 \times 25 \times 26.5$  cm) supplied with a roll of cotton saturated with a 10% solution of sugar water. No significant differences were observed in adult

emergence or survival between the test volumes and the controls.

Tests were also conducted to determine the feasibility of storing *Cx. quinquefasciatus* eggs. Eggs that had been dried and separated were stored dry in glass vials at  $10-12^\circ\text{C}$  or at room temperature ( $21-25^\circ\text{C}$ ), and undried eggs were stored intact on water at  $10-12^\circ\text{C}$ . We found, as did Singh et al. (1975), that when intact rafts were stored at  $10-12^\circ\text{C}$  the hatch remained above 80% for 7 days. Dried eggs, however, dropped below 70% hatch after 3 days at  $10-12^\circ\text{C}$ , and when held at room temperature only 35% hatched. *Culex quinquefasciatus* eggs appear to have minimal resistance to prolonged desiccation; however, short-term drying for volumetric measurement had no effect on hatchability.

The success of this new technique has greatly improved our capability of rearing *Cx. quinquefasciatus*, and 0.04 ml of dried eggs is now the standard set for routine colony maintenance. Development of the immature stages has been much more synchronous, and product variability has been greatly reduced. We are currently assessing this drying procedure with *Culex salinarius* Coquillett with preliminary results similar to those reported in this paper. This technique could possibly also be used for rearing other *Culex* species.

#### References Cited

- Bailey, D. L., J. A. Thomas, W. L. Munroe and D. A. Dame. 1979. Viability of eggs of *Anopheles albimanus* and *Anopheles quadrimaculatus* when dried and stored at various temperatures. Mosq. News 39:111-113.
- Bailey, D. L., R. E. Lowe, D. A. Dame and J. A. Seawright. 1980. Mass rearing the genetically altered Macho strain of *Anopheles albimanus* Wiedemann. Am. J. Trop. Med. Hyg. 29:141-199.
- Dame, D. A., D. G. Haile, C. S. Lofgren, D. L. Bailey and W. L. Munroe. 1978. Improved rearing techniques for larval *Anopheles albimanus*: Use of dried mosquito eggs and electric heating tapes. Mosq. News 38:68-74.

- Hazard, E. I. 1967. Modification of the ice water method for harvesting *Anopheles* and *Culex* pupae. *Mosq. News* 27:115-116.
- Singh, K. R. P., R. S. Patterson, G. C. LaBrecque and R. K. Razdan. 1975. Mass rearing of *Culex pipiens fatigans* Wied. *J. Commun. Dis.* 7:31-53.

# A FIELD TRIAL OF EXPANDED POLYSTYRENE BALLS FOR THE CONTROL OF *CULEX* MOSQUITOES BREEDING IN PIT LATRINES

PAUL REITER<sup>1, 2</sup>

Pit latrines are frequently a prolific breeding site for *Culex quinquefasciatus* Say. In many countries the construction of large numbers of latrines, often as a component of public health programs, has contributed to the burgeoning problem of urban filariasis (Southgate 1984, WHO 1984).

Mosquito-infested latrines present a difficult control problem. Conventional methods, using insecticides or larvicidal oils, have given good results, but are expensive, require frequent application on a routine basis, and may be hampered by problems of resistance (World Health Organization 1984). As an alternative, Reiter (1978) suggested that a layer of expanded polystyrene balls (EPB) could be used as a floating "blanket" to prevent mosquitoes from breeding in these sites. Recent field-trials of the idea have given encouraging results in Tanzania and Kenya (Curtis and Minjas 1985) and in Zimbabwe (Morgan and Mara 1982). In this note I report on a small trial which I conducted in Kisumu, Kenya, in 1977.

The trial area included low-lying parts of the Nyalenda and Nyamasaria neighborhoods of Kisumu (0° 06'S, 34° 45' E), on the shores of Lake Nyanza (formerly Lake Victoria). During the rainy season, the water table in these districts is higher than the bottom of many of the latrine pits, providing ideal conditions for *Cx. quinquefasciatus* and other sewage-breeding Diptera.

Mosquito production was monitored by trapping the departing adult insects in a window exit-trap (Service 1963) which was placed over the drop-hole before sunset and left there overnight. A skirt of denim cloth sewn around the entrance of each trap formed a seal between the trap and the latrine platform. Captured insects were killed in the traps with a pyrethrum spray and transferred to cartons for transport to the laboratory. On trap-nights, owners agreed to use latrines belonging to neighbors.

Sixteen latrines were chosen for the trial. Eight of these were randomly selected for treatment, and the remainder used as untreated controls. Expanded polystyrene balls with a mean diameter of about 0.4 cm (manufactured by BASF GmbH, and donated by Booth's Manufacturing (Africa) Ltd., Nairobi)<sup>3</sup> were scattered by hand to a depth of 5 cm (2 inches) in each treatment latrine. All latrines were monitored on 31 nights from mid-May through mid-August.

For 2 to 4 nights after the treatment, large numbers of mosquitoes continued to emerge from the treated latrines. Following this, however, there was a dramatic change; on 28 collection nights from day 7 to day 86, the mean count (female, non-gravid) for treated latrines was less than 2% of the mean for the controls (Table 1). The highest number of nongravid mosquitoes per treated latrine per night was 52, whereas from the untreated latrines it was 5,325.

Laboratory observations (Reiter 1978) had

Table 1. Treatment of pit latrines with expanded polystyrene balls. Mean numbers of adult mosquitoes (female, nongravid) per trap-night. Twenty-six trap nights from night 6 through night 86 after treatment.

Latrine number	Mean mosquitoes per trap night ( $\pm$ s.e. of mean)	
	Treated	Untreated
1	1.2 (0.2)	324.3 (63.6)
2	7.4 (1.4)	123.8 (24.3)
3	3.4 (0.7)	168.4 (33.7)
4	2.3 (0.6) <sup>1</sup>	177.3 (34.8)
5	6.5 (1.3)	236.3 (46.3)
6	4.5 (0.9)	161.9 (31.8)
7	6.6 (1.3)	836.4 (164.0)
8	— <sup>2</sup>	642.8 (126.1)
Mean	6.1	33.9

<sup>1</sup> Studies completed while the author was at the Department of Chemistry, The University, Southampton, United Kingdom, SO9 5NH.

<sup>2</sup> Dengue Branch, Division of Vector-Borne Viral Diseases, Center for Infectious Diseases, Centers for Disease Control, U. S. Public Health Service, Department of Health and Human Services, G. P. O. Box 4532, San Juan, Puerto Rico 00936.

<sup>3</sup> Latrine collapsed on day 56.

<sup>2</sup> Latrine collapsed on day 15.

<sup>3</sup> The use of trade names or commercial sources is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.